Overview

Product name: Anti-eIF4E (phospho S209) antibody [EP2151Y]
Description: Rabbit monoclonal [EP2151Y] to eIF4E (phospho S209)
Host species: Rabbit
Tested applications: Suitable for: ICC/IF, WB, IP, IHC-P, Dot blot
Species reactivity: Reacts with: Mouse, Rat, Human, Pig
Immunogen: Synthetic peptide within Human eIF4E. The exact sequence is proprietary.
Database link: P06730
Positive control: WB: 293 cell lysate treated with alkaline phosphatase and HEK293 cell lysate treated with Dexamethasone. IHC-P: human breast carcinoma tissue. ICC/IF: HEK293 cells.
General notes: This product is a recombinant monoclonal antibody, which offers several advantages including:
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production
For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

Properties

Form: Liquid
Storage instructions: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer: pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity
Protein A purified

Clonality
Monoclonal

Clone number
EP2151Y

Isotype
IgG

Applications

The Abpromise guarantee
Our Abpromise guarantee covers the use of ab76256 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td></td>
<td>1/500.</td>
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<tr>
<td>WB</td>
<td></td>
<td>1/1000 - 1/100000. Detects a band of approximately 25 kDa (predicted molecular weight: 25 kDa).</td>
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<tr>
<td>IP</td>
<td></td>
<td>1/40 - 1/60.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★★★ (1)</td>
<td>1/50 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.</td>
</tr>
<tr>
<td>Dot blot</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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Target

Function
Its translation stimulation activity is repressed by binding to the complex CYFIP1-FMR1 (By similarity). Recognizes and binds the 7-methylguanosine-containing mRNA cap during an early step in the initiation of protein synthesis and facilitates ribosome binding by inducing the unwinding of the mRNAs secondary structures. Component of the CYFIP1-EIF4E-FMR1 complex which binds to the mRNA cap and mediates translational repression. In the CYFIP1-EIF4E-FMR1 complex this subunit mediates the binding to the mRNA cap.

Sequence similarities
Belongs to the eukaryotic initiation factor 4E family.

Post-translational modifications
Phosphorylation increases the ability of the protein to bind to mRNA caps and to form the eIF4F complex.
Western blot - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

All lanes: Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256) at 1/100000 dilution (purified)

Lane 1: Untreated HEK293 whole cell lysate
Lane 2: HEK293 cells treated with 10uM dexamethasone for 1 hour whole cell lysate
Lane 3: HEK293 cells treated with 10uM dexamethasone for 1 hour whole cell lysate. The membrane was then incubated with alkaline phosphatase.

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 25 kDa
Observed band size: 25 kDa

Exposure time: 30 seconds

Blocking buffer and concentration 2% BSA/TBST.
Diluting buffer and concentration 2% BSA/TBST.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labelling eIF4E with purified ab76256 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a goat anti-rabbit IgG H&L (HRP) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Immunocytochemistry/Immunofluorescence analysis of untreated, 20% serum treated and 20% serum + LP treated NIH/3T3 cells labelling eIF4E (phospho S209) with ab76256 (left) and eIF4E with ab33766 (right) both at a dilution of 1/500. Cells were fixed with 100% methanol. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used. The image shows increased cytoplasmic staining after 20% serum treatment on NIH3T3 cells when compared with no serum treated cells. The LP treatment decreased the increased cytoplasmic staining caused by 20% serum. ab33766 was used as a Pan control for ab76256. The results showed cytoplasmic staining on no serum, 20% serum and 20% serum +LP treated NIH3T3 cells.
Immunoprecipitation - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

ab76256 (purified) at 1/40 immunoprecipitating eIF4E (phospho S209) in HEK293 whole cell lysate. 10 µg of cell lysate was present in the input. For western blotting, a HRP-conjugated VeriBlot for IP Detection Reagent (ab131366) (1/1,500) was used for detection. A rabbit monoclonal IgG (ab172730) was used instead of ab128913 as a negative control (Lane 3).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Western blot - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

All lanes: Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256) at 1/1000 dilution (purified)

Lane 1: Mouse spleen lysate
Lane 2: Rat brain lysate
Lane 3: Pig heart lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 25 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.
Dot blot analysis of eIF4E (pS209) peptide (Lane 1) and eIF4E non-phospho peptide (Lane 2) labelling eIF4E (pS209) with purified ab76256 at a dilution of 1/1000. **ab97051** (Peroxidase conjugated goat anti-rabbit IgG (H+L)) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

Immunocytochemistry/Immunofluorescence analysis of serum starved HEK293 cells treated with CGP 57380 **ab120365** labelling eIF4E (phospho S209) with unpurified **ab32124** at 1/100. Decrease in eIF4E (phospho S209) expression correlates with increased concentration of CGP 57380, as described in literature.

The cells were incubated at 37°C for 1h in media containing different concentrations of **ab120365** (CGP 57380) in DMSO, fixed with 100% methanol for 5 minutes at -20°C and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with unpurified ab76256 was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (**ab96899**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.
All lanes: Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256) at 1/50000 dilution (purified)

Lane 1: Untreated HEK293 cell lysate
Lane 2: HEK293 treated with 10mM Dexamethasone 1 hour lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 25 kDa

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.

All lanes: Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256) at 1/50000 dilution (purified)

Lane 1: Untreated 293 cell lysate
Lane 2: 293 cell lysate treated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 25 kDa

Exposure time: 1 minute

Blocking and dilution buffer: 5% NFDM/TBST.
**Western blot** - Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)

**All lanes**: Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256) at 1/100000 dilution (purified)

**Lane 1**: Untreated HEK293 cell lysate

**Lane 2**: HEK293 cell lysate - treated with Dexamethasone

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

**Predicted band size**: 25 kDa

Exposure time:

eIF4E pS209: 15 seconds.
eIF4E: 3 minutes.

Blocking and dilution buffer: 5% NFDM/TBST.

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**Why choose a recombinant antibody?**

- **Research with confidence**
  - Consistent and reproducible results

- **Long-term and scalable supply**
  - Recombinant technology

- **Success from the first experiment**
  - Confirmed specificity

- **Ethical standards compliant**

*Anti-eIF4E (phospho S209) antibody [EP2151Y] (ab76256)*

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**Please note**: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"
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